

DTIC
ELECTE
SEP 23 1993
S E D

[³H]-SAXITOXINOL METABOLISM AND ELIMINATION IN THE RAT

HARRY B. HINES, SYED M. NASEEM and ROBERT W. WANNEMACHER JR

Pathophysiology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701-5011, U.S.A.

(Received 8 April 1992; accepted 5 January 1993)

H. B. HINES, S. M. NASEEM and R. W. WANNEMACHER JR. [³H]-Saxitoxinol metabolism and elimination in the rat. *Toxicol* 31, 905-908, 1993.—Tritiated saxitoxinol was used to obtain preliminary information on saxitoxin metabolism in the rat. Sublethal doses of tritiated saxitoxinol (18.9- μ Ci/kg; 3.8 μ g/kg) were injected i.v. into each of six rats. Urine and fecal samples were collected up to 144 hr post-injection. Within 4 hr, 60% of injected radioactivity was excreted in urine. No radioactivity was found in feces. High performance liquid chromatography analyses of urine showed that saxitoxinol was not metabolized by the rats.

CLINICIANS have observed that, if patients afflicted with paralytic shellfish poisoning (PSP) survive 24 hr, either with or without mechanical ventilation, chances for a rapid and full recovery are excellent (MEYER, 1953; ACRES and GRAY, 1978; SAKAMOTO *et al.*, 1987). Such observations suggest that toxin(s) responsible for PSP undergo either rapid excretion, metabolism or both; but there are no known studies of PSP toxin metabolism in mammals.

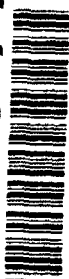
We used tritiated, reduced saxitoxin (dihydrosaxitoxin or saxitoxinol; [³H]-STXOL) to study PSP metabolism in mammals. Saxitoxinol is an analog of saxitoxin (STX), which is the most studied PSP toxin and one of the most toxic. Saxitoxinol retains most of STX's structural features and STX's ionic characteristics, although two epimers are produced upon STX reduction with borohydride (SHIMIZU *et al.*, 1981).

Purified, tritiated STXOL (specific activity 1.4 Ci/mmol), prepared according to the method of SHIMIZU *et al.* (1981), was provided by Dr FUN SUN CHU of the University of Wisconsin, Madison, WI, U.S.A. A STXOL dose of 18.9 μ Ci/kg (3.8 μ g/kg) was injected into the penile vein of each of six male Fischer VAF/Plus rats (Charles River, Inc., Wilmington, MA, U.S.A.) weighing approximately 195 g each and housed individually in metabolism cages. One ml of 1.0 M acetic acid was added to each urine collection vial to prevent pH-related degradation (MEYER, 1953; SHIMIZU, 1988) and to inhibit bacterial growth. Urine and fecal samples were collected at 4, 24, 48, 72 and 144 hr post-injection. A 0.1 ml aliquot of each urine sample was assayed for total radioactivity. The remainder was frozen at -20°C until analyzed by high performance liquid chromatography (HPLC). Fecal samples were placed in 5 ml of 0.01 M acetic acid, homogenized and centrifuged at 3500 $\times g$ for 10 min. A 1.0 ml aliquot of supernatant was counted for total radioactivity.



498

AD-A269 712



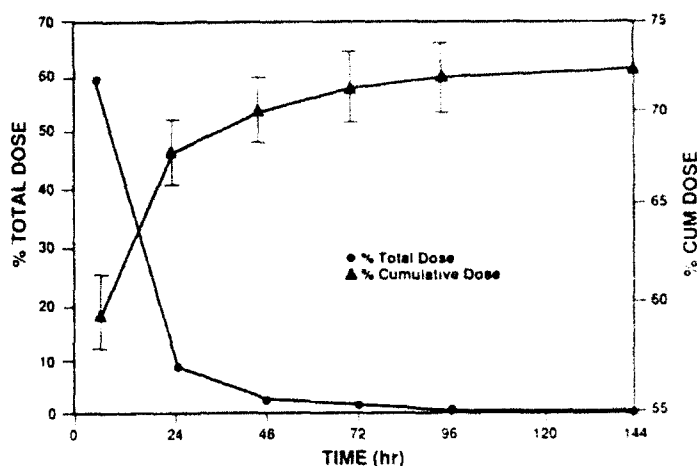


FIG. 1. RAT URINARY EXCRETION PROFILE OF RADIOACTIVITY EXPRESSED AS PERCENTAGE OF TOTAL DOSE AND PERCENTAGE OF CUMULATIVE DOSE.

All points are the averages of six measurements (mean \pm S.E.M.). Toxicokinetic analysis yielded the following values: $T_{1/2e} = 12.3$ hr, α phase = 0.0563.

Rat urine (1.0 ml) was filtered (0.22 μ m filter), and 100 μ l was injected into a Perkin-Elmer Series 4 liquid chromatograph fitted with a 150 \times 4.1 mm PRP-1 column (Hamilton, Inc., Reno, NV, U.S.A.). Chromatography conditions and mobile phases have been described (SULLIVAN and IWAOKA, 1983). A post-column system was not used. One-minute fractions were collected for scintillation counting.

Both curves shown in Fig. 1 depict the rapid excretion of radioactivity in urine. No radioactivity was detectable in feces at any time. By 4 hr post-injection, 60% of the injected radioactivity was excreted in urine (Fig. 1). Rapid urinary elimination slowed by 48 hr, and negligible additional radioactivity was excreted throughout the remainder of the experiment, which was terminated at 144 hr. Approximately 72% of the injected dose was excreted by 144 hr, while 28% remained in rat tissues. Toxin remaining in the rat may have been retained either in an intact or altered form. Analysis by HPLC revealed that, for each rat, there was only one radioactive peak in urine for each collection period. The peak was identified as parent by co-injection of standard [3 H]-STXOL. Generally, peak retention was consistent for many analyses. Column performance changed slightly after repeated urine injections but was restored with extensive washing with elution buffer. Identification and retention consistency are illustrated in Fig. 2 (A, B and C). Figure 2D depicts the slight change in column performance which caused a minor retention shift. Attempts to confirm the peak's identity by fast atom bombardment mass spectrometry were unsuccessful due to ion suppression caused by urine components that co-eluted with STXOL in the purification process. Efforts are continuing to improve the isolation/purification procedure.

The urinary excretion profile supports clinical observations of rapid recovery from PSP in non-lethal cases. Species and route of administration differ (intravenous vs. oral), however, and do not permit direct comparisons. Our analyses indicated that excreted toxin was not metabolized by the rats, although approximately 28% of the injected radioactivity was not recovered.

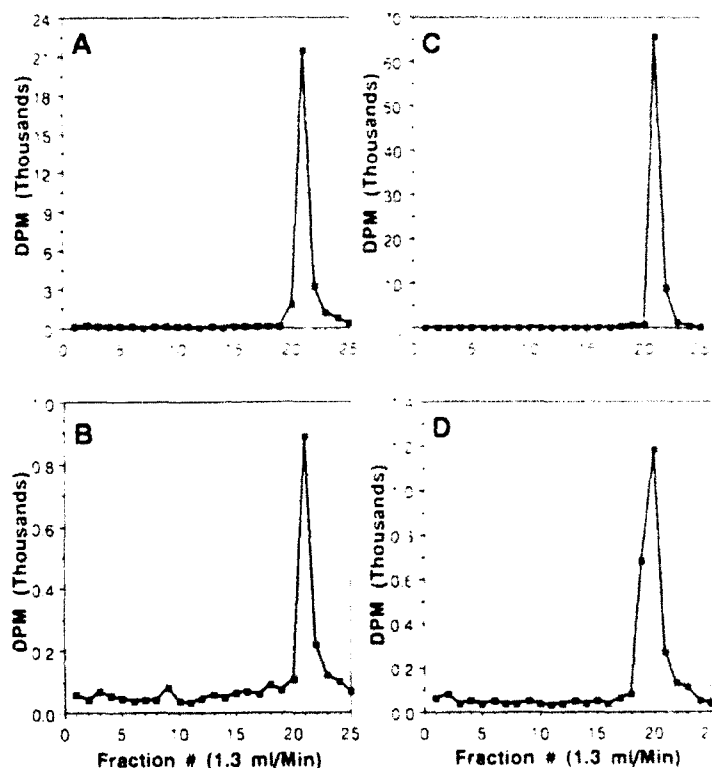


FIG. 2. REPRESENTATIVE HPLC RADIOCHROMATOGRAMS OF INDIVIDUAL RAT URINE SAMPLES. The large peak appearing in each radiochromatogram was identified as [^3H]-saxitoxinol by coinjection analysis. (A) Standard [^3H]-saxitoxinol spiked into acidified rat urine (pH 5.0) and incubated at 37°C for 144 hr. Saxitoxinol radioactivity peaked in fraction 21. (B) Individual rat urine sample collected at 144 hr. Saxitoxinol radioactivity peaked in fraction 21. (C) Individual rat urine sample collected at 4 hr. Saxitoxinol radioactivity peaked in fraction 21. (D) Individual rat urine sample collected at 144 hr. Saxitoxinol radioactivity peaked in fraction 20 due to changed column performance.

From these data, we postulate that recovery from PSP caused by STX generally involves rapid excretion of parent toxin. Studies with guinea-pigs injected with toxic amounts of STX also support this postulate. Time to return of spontaneous ventilation was dose dependent if guinea-pigs were mechanically ventilated while spontaneous respiration was blocked (MILLER and FRANZ, 1989). To substantiate these results, studies of unaltered PSP toxins, such as STX, and oral administration (or human ingestion) will be required. Better analytical techniques and/or stable radiolabels for PSP toxins are needed. These investigations are in progress.

Acknowledgements—The authors thank Dr JUDITH G. PACE for assistance with toxicokinetics analysis and Mr RICHARD DINTERMAN and Mr KENNETH HOY for technical assistance. The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense. The experiments conducted herein were performed according to the principles set forth in the current edition of the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

REFERENCES

- ACRES, J. and GRAY, J. (1978) Paralytic shellfish poisoning. *Can. Med. Assoc. J.* **119**, 1195-1197.
- MEYER, K. F. (1953) Food poisoning. *N. Eng. J. Med.* **249**, 843-852.
- MILLER, T. and FRANZ, D. (1989) Effect of mechanical ventilation on saxitoxin intoxication in guinea pigs. *FASEB J.* **3**, A688.
- SAKAMOTO, Y., LOCKEY, R. F. and KRZANOWSKI, J. J., JR (1987) Shellfish and fish poisoning related to the toxic dinoflagellates. *South. Med. J.* **80**, 866-872.
- SHIMIZU, Y. (1988) The chemistry of paralytic shellfish toxins. In: *Handbook of Natural Toxins*, Vol. 3, pp. 63-85 (TU, A. T., Ed.). New York: Marcel Dekker.
- SHIMIZU, Y., HSU, C. P. and GRENEAK, A. (1981) Structure of saxitoxin in solution and stereochemistry of dihydrosaxitoxin. *J. Am. chem. Soc.* **103**, 605-609.
- SULLIVAN, J. J. and IWAOKA, W. T. (1983) High pressure liquid chromatographic determination of toxins associated with paralytic shellfish poisoning. *J. Assoc. Off. Anal. Chem.* **66**, 297-303.

Accession For	
NTIS CRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input checked="" type="checkbox"/>
U announced	<input type="checkbox"/>
Justification	
By	
Distribution /	
Availability Codes	
Dist	Avail and/or Special
A-1	20

DTIC QUALITY INSPECTED 1